E.D. R

Vol. 106. Part 11. Pp. 421–437. 5 figs. 12th October, 1955.

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THE TRANSACTIONS OF THE ROYAL ENTOMOLOGICAL SOCIETY OF LONDON

World List abbreviation: *Trans. R. ent. Soc. Lond.*

CONTENTS

PAGE

Long, D. B., MA., Ph.D., F.R.E.S. Observations on sub-social behaviour in two species of Lepidopterous larvae, *Pieris brassicae* L., and *Plusia gamma* L. . . . . 421–437, 5 figs.

LONDON:
PUBLISHED BY THE SOCIETY AND
SOLD AT ITS ROOMS, 41, QUEEN'S GATE, S.W. 7

Price 6s. 0d.

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OBSERVATIONS ON SUB-SOCIAL BEHAVIOUR IN TWO SPECIES OF LEPIDOPTEROUS LARVAE, *PIERIS BRASSICAE* L. AND *PLUSIA GAMMA* L.

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Manuscript received 31st January, 1955.

(Read 4th May, 1955.)

With 5 Text-figures.

Contents.

PAGE

Introduction 421

Pieris brassicae L.

Formation and Maintenance of the Aggregation 422
Mass Feeding Rhythms 423
Dissolution of the Aggregation 424
The Aggregating Ability of Individual Larvae throughout Life . 425

Plusia gamma L.

The Senses and the Effects of Association 425
The Aggregation of the Larvae 427
Observations on an Aggregation in the Field 431
The Effect of the Number of Larvae in Association . . . 433

Discussion 434
Summary 436
Acknowledgements 437
References 437

Introduction.

The social behaviour of certain insects such as ants, bees, wasps, etc., has attracted in the past considerable attention. However, this high order of behaviour is restricted to very few species, the insect world in general existing in states from pure solitariness to that of sub-social aggregation. Lepidopterous larvae afford many examples covering the extremes of this range. Those which lead a solitary existence generally do so as the result of the habit of the adult in laying eggs singly or in pairs rather than a distinct anti-social facet in the larval behaviour. In other cases where eggs may be loosely scattered or laid in tight clusters the opportunity for aggregation will exist. Variation in the subsequent aggregation may be illustrated by the loose associations of the larvae of *Plusia gamma* in contrast to the closely packed aggregations of *Pieris*

brassicae. In the following paper it is proposed to consider some of the facets of sub-social behaviour concerned with aggregation in the two species of caterpillars and some of the subsequent effects.

Formation and Maintenance of the Aggregation in *P. brassicae*.

The young larvae of the Large White butterfly (*P. brassicae*) most frequently may be found in the second brood during July in large tightly packed groups on the underside of cabbage leaves. By early August the groups will be found to have split up and these older larvae wander over the plants causing extensive damage. In the latter part of August the fully grown larvae start to leave the plants prior to pupation.

It has been repeatedly observed that shortly after a cluster of eggs begins to hatch virtually all the young larvae have aggregated on a patch of leaf to one side of the egg site. As the ensuing mass behaviour depended to a large extent on the simultaneity of the hatching and the successful aggregations of the young larvae, a study was made on the processes which brought this about.

The eggs of *P. brassicae* have been found to be laid in tight clusters of up to 70 or more eggs. The first larvae hatched by eating their way out of the top of the egg shell, after which climbing out on top of the adjacent eggs and turning round as they did so they continued to eat the remains of their own shells. When all but the base of the shell had been eaten they turned their attention to the tops of adjacent eggs which they began to eat. This served to assist in the release of the contained larvae which rapidly emerged and also began to feed on the shell of its own egg and those adjacent to it. This pattern of events resulted in the rapid hatching of entire egg clusters.

The next feature to appear was that of silk spinning and this appeared to be of prime importance in the formation and the subsequent maintenance of the aggregation. As the bulk of the egg cluster hatched the first larvae to emerge stopped feeding on the egg shells and began to wander off the top of the egg mass down on to the adjacent leaf surface spinning a chain of silk web by rhythmic side to side movements of the head as they crawled (fig. 1). Successive larvae

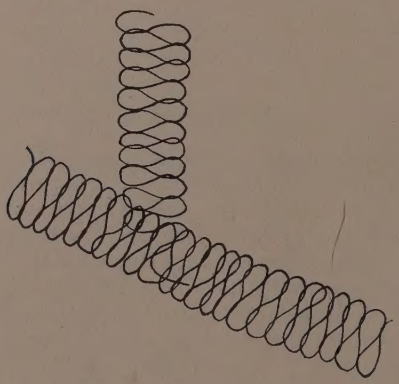

Fig. 1.—Silk trails spun by crawling larvae.

tended to follow this silken trail and so were led to the area where the first larvae had settled. Symczak (1950) considered that the ability of wandering larvae to follow previous paths was due to a sense of smell but no account was taken of the silk spinning habit. The spinning of larvae as they aggregated resulted in the area being covered with a continuous silken mat. Once this mat had been formed further spinning generally was restricted to fresh areas of leaf surface at the edge of the mat which was in this way continually increasing in size.

The Mass Feeding Rhythms of *P. brassicae*.

Aggregations of larvae have been observed to have a distinct form of rhythm in their feeding periods. The entire mass of larvae alternated between periods of activity, feeding at a common feeding site, and apparent inactivity in an area a small distance away from this site. A similar feeding rhythm has been observed by Roche (1954) in the larvae of another member of the Pieridae, *Mylothris chloris*. In this case the cycle was diurnal and the distance travelled much greater, though the operating factors were probably much the same. The larval resting site was at the base of the tree where the larvae aggregated from dawn until dusk. They then moved in procession to the feeding site, thought to be at the top of the tree, and did not return to the resting site until the following dawn.

The " feeding " rhythm of *P. brassicae* was established by a few of the earlier larvae which had proceeded to the further edge of the silk mat and begun to eat a hole in the leaf by the time the last larvae were joining the aggregation. The feeding larvae were rapidly joined by others, which extended the silk mat round the enlarging feeding hole. The mat was still further extended by later larvae wandering round the edge of the ring of the larvae now surrounding the feeding hole. Larvae which finished feeding moved backwards out of the ring of larvae and were rapidly replaced by larvae which had not yet fed. The enlargement of the hole also permitted additional larvae to feed. During feeding both the leaf and the attached silken mat were eaten away together.

The first larvae to finish feeding would move away from the feeding site back on to the deserted silk mat and there settled in close aggregation. Successive larvae surrounded the initial group as they settled. After a quiescent and somewhat variable period of about five minutes, though occasionally up to ten minutes, the original larvae would begin to move and to push their way out of the mass in returning to the feeding hole. The movement of these larvae appeared to stimulate the other larvae into activity and within a short time the entire mass would be active again and beginning to feed.

The mass behaviour thus appeared to depend on a few initiating larvae and their ability to transmit a sense of their activity to other larvae. This ability was due to the fact that the larvae sought the close proximity of their neighbours. Thus, movement of an individual larva together with a sense of its direction was conveyed to adjacent larvae either by direct contact between the larval skins or by movement between the interlocked lateral hairs. Once stimulated the adjacent larvae would begin to move, transmitting in their turn a sense of their activity. Thus a wave of activity would spread through the larval mass. The direction of the general movement frequently followed that of the initiating larvae. Larvae not following the direction of the general mass were frequently

observed to turn in that direction, presumably being influenced by the setal stimulation caused by passing larvae.

In the feeding rhythm two other factors came into play. The actual time spent feeding and resting was probably physiologically determined in the initiating larvae, depending on the time required to ingest and digest a certain quantity of food such as could result, for instance, by the filling and the emptying of the crop. When about to feed their subsequent movement served as the necessary stimulus for movement in the rest of the larvae which, due to the simultaneity of the hatching, were in a similar physiological state in terms of the times required for resting and feeding. However, there must have existed some inherent cycle of activity in the order—feeding, movement, resting, movement, feeding—which induced the initiating larvae to go to and from the feeding site ; as the same behaviour appears with solitary larvae. Whether larvae were stimulated to feed by the freshly cut leaf surface at the feeding hole was not determined, but they appeared to be able to transmit a sense of their feeding activity to other larvae wandering aimlessly round them. This was due to the fact that, as the larvae chewed the leaf away, they developed a series of small rhythmic contractions along their bodies which were quite unlike those involved in locomotion. It was observed that wandering larvae on coming into contact with the tail ends of feeding larvae would immediately make determined efforts to squeeze between them and on reaching the cut leaf surface would then begin to feed. The feeding rhythm itself was a tenuous one which could easily be upset by any major disturbance of the larval mass. In such circumstances the feeding relays would split up into two or more fractions, so that there would be a continual succession of larvae going to and from the feeding site and the whole picture would become confused. After some time a unified mass rhythm might reform, but frequently the aggregation would divide in both the matter of the resting and the feeding sites, fresh silk mats and feeding holes being formed. From this it would appear that a unified mass behaviour was essential for the maintenance of the aggregation.

The Dissolution of the Aggregation (*P. brassicae*).

The original aggregation was generally found to be maintained throughout the first one or two instars. As the feeding hole enlarged it frequently happened that a large vein would be encountered, a new feeding hole would be started on the other side of it and the aggregation would be divided about the vein. Occasionally a new feeding hole would be started at another edge of the silk mat and this would result in a division of the aggregation, though this was more usually the result of some form of disturbance. By the fourth instar the aggregation would have split up into a number of groups of 10–20 larvae each. As the destruction of the food leaf increased, larvae would wander off this leaf on to other leaves and there begin to feed on the leaf edges. These larvae continued to show on an individual level a feeding rhythm, resting a short distance away from the feeding site. On the new leaves small groups would form, but these seldom persisted through into the last instar as much more than pairs, probably due to the fact that the feeding rhythms of all the individuals forming a group did not coincide and the larvae thus become separated by their greater capacity to wander. It should be noted that the feature of silk spinning was present throughout the

larval life, but with increasing weight the larvae tended to move on to the upper side of the leaf falling more easily off the underside when disturbed. When feeding finally ceases at the end of the fifth instar the capacity for wandering is shown by the known distances these larvae will travel in order to find a suitable pupation site in which to over-winter.

The Aggregating Ability of Individual Larvae Throughout Life.

To study this factor larvae were reared under solitary conditions and their reactions observed at different periods of their life when solitary larvae are released together on to a fresh leaf. In the first and second instar aggregation depended largely on the larvae striking a silk trail which would bring them together in the manner previously discussed. In this way solitary larvae could be led to join an already established aggregation provided the individuals in the latter were of the equivalent order of size. By the third instar however solitary larvae showed a different reaction to each other. Within an established aggregation there was an essential quality of mutual passiveness between larvae, but solitary larvae showed investigatory movements on meeting which were accompanied by a rapid flick type reaction with the head and fore part of the body. This tended to interfere with the normal pattern of maintaining a close aggregation. However, when released with established aggregations they associated with the edge of the aggregations, but 18 hours later their feeding rhythms had not coincided. By the fourth instar the individual pattern of behaviour appeared to be too strongly established to permit larvae to form an aggregation. In this instar the larvae were becoming increasingly active, although the tendency to aggregate was still present and larvae would pair, but soon separated again and appeared incapable of maintaining even a small aggregation, nor would they join an established aggregation. From this it would appear that, although an aggregating tendency may exist throughout most of the larval life, the successful realisation of this tendency involves a complexity of physiological and psychological factors which are normally co-ordinated in the early rapid formation of the initial aggregation.

The Senses and the Effects of Association in *P. gamma*.

In the larvae of *P. gamma* diverse and extensive effects have been shown as the result of the population density (Long, 1953). Amongst these effects, that of colour changes was considered in some detail. It was found that numbers of larvae kept together during the period of their development, although variable in colour, were darker than their solitary counterparts and a colour scale was devised to estimate the degree of darkness in which a series of grades of progressive darkening were designated as colour groups 1–5. The effect did not depend on a sense of sight but was probably either the result of physical contact or a gas produced by the larval mass. An experiment was therefore designed to separate these factors and carried out in duplicate. A crowded culture of 60 and a number of solitary larvae were set up and bred on agricultural mustard as previously described (Long, 1953). When the cultures were well into the third instar larvae were transferred to small cages covered with muslin and containing a leaf. Some of the cages were set up in pairs and surrounded by crowded cultures of 60 larvae and their food material in 2 litre breeding jars.

Other cages were first mounted in a tube of fine mesh Tygan[1] before setting up in the crowded cultures (fig. 2). Other solitary larvae were set up in breeding jars fitted with gas-tight rubber covers and filled with air containing 5 per cent. carbon dioxide, whilst the remainder were kept as controls. As the larvae grew

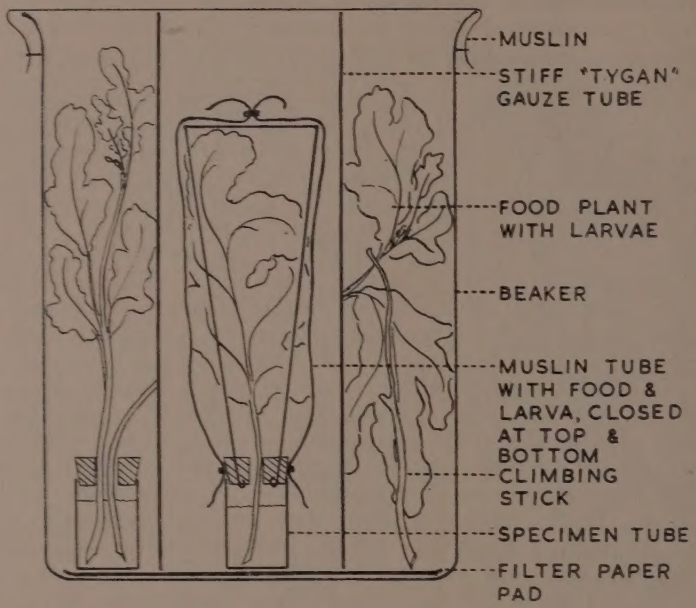

FIG. 2.—Isolation of a larva in a crowded breeding container.

larger the number of larvae in the crowded cultures was progressively reduced from 60 to 40. The colours of the larvae in their final instar were noted and the results are given in Table I.

TABLE I.—*The effect of gas and larval contact on the colour of solitary larvae of* P. gamma.

Condition.	Number set up.	Colour Group. 1	2	3	4	5	Mean colour.
Solitary control .	20	18	.	.	.	.	1·0
Carbon dioxide .	10	8	.	.	.	.	1·0
Double walled cages .	20	17	2	.	.	.	1·1
Single walled cages .	20	4	2	4	7	.	2·8
Crowd control . .	60	3	6	23	25	1	3·3

It can be seen in this table that neither carbon dioxide nor any other gas associated with the crowded culture, which would diffuse through the double walled cages, affected the colour of the solitary larvae. Thus it appears that

[1] A proprietary brand of nylon mesh.

the darkening of colour in a crowded culture is not attributable to a sense of smell nor a gas. The larvae in the single walled cages, however, showed a marked departure from the solitary controls, with a colour group mean closely approaching that of the crowd control. Unlike the other larvae, these larvae could experience a limited degree of contact through the soft walls of the cage so that a sense of contact must be considered responsible for the effects noted. In view of earlier findings on the effect of a limited period of exposure to crowded conditions (Long, 1953) it is not surprising that these larvae did not attain the same degree of darkening as occurred in the crowded control; in fact it is remarkable that with such a limited degree of contact the effect should have been so pronounced.

It had been earlier observed that the duration of the larval stage in crowded cultures of *P. gamma* was less than that of solitary cultures and it is interesting to note that the larvae in the single walled cages took 92 per cent of the time taken by the non-affected larvae in the double walled cages to complete their development. A similar figure was obtained in an earlier experiment in which larvae were changed from solitary to crowded conditions for an equivalent period (Long, 1953). From this it appears that larval contact is responsible for inducing both the colour change and the faster rate of development associated with crowded conditions. In the field the opportunities for frequent larval contact principally depend on the existence of some form of aggregation the nature of which is considered in a later section.

The Aggregation of the Larvae of *P. gamma*.

The larvae of the Silver Y moth (*Plusia gamma*) are normally most abundant from mid-summer onwards and may be found on the undersides of a variety of low growing plants, including brassicas, clover, nettles, thistles, etc. The numbers of larvae are extremely variable and may be found singly on some occasions, whilst on others they may be scattered in loose groups over one or more leaves of the food-plant. When the larvae are fully grown they spin silk cocoons in which they pupate on the plant.

Unlike the eggs of *P. brassicae*, which are laid in tight clusters, the eggs of *P. gamma* are laid singly dotted about the underside of the lower leaves of the food-plants. The variable manner in which the adult females lay their eggs no doubt accounts to some extent for the variable number of larvae which may be found on any one plant, though this is also affected by the number of laying females present. Under experimental conditions it was found that a single laying of eggs would all hatch within a period of one day, so that the opportunity of the resulting larvae to aggregate could occur. Experiments were carried out to determine the nature of the aggregation.

In the first series the floor of a glass circular trough of 11 in. diameter was lined with paper marked off radially into twelve equal areas. The centre area of 3 in. diameter was excluded from the arena by a smaller circular glass trough. A 75 watt lamp was hung centrally 3 ft. above the trough, which was covered with a ground glass screen. Twenty-four larvae were released evenly over the floor of the arena and an initial period of 5–15 minutes allowed for the larvae to settle down. Observations were then made of the dispersion of the larvae over the areas at two and a half minute intervals for a period of one hour. The

variation of temperature over the experimental periods was observed not to exceed 0·5° C.

A statistical analysis of the results failed to show any significant evidence of aggregation under these conditions. It was observed that, as the experiment proceeded, the larvae became increasingly active and were almost continually on the move. From this it was concluded that, because the larvae were not on their normal plant substrate, their food searching behaviour became dominant and their wanderings were accordingly random, and did not necessarily reflect a truly non-gregarious tendency.

A similar series of experiments was attempted by marking out equal areas on a horizontal dandelion leaf and observing the dispersion of larvae at 30-minute intervals for 5-hour periods. The results, however, were inconclusive and it was considered that this method of approach was unsuitable for studying the loose aggregations of *P. gamma*. The chief difficulty was that of the size of the unit areas—if they were large they interfered with the results, for the larvae must cover large distances before an opportunity of meeting occurred, whilst if they were small enough to reduce this factor to practical limits from the experimental point of view, it frequently happened that a group of larvae would be situated over the boundaries of two or more areas and so did not appear as such in the results. Furthermore, the situation of the larger veins and the leaf edges, together with other factors, influenced the movements of the larvae, which made a consideration of their dispersion in relation to the total leaf area unreliable.

A new approach was therefore made in the final series of experiments in which the orbits of influence of the individual larvae were considered as the basic unit. Unlike the previous method this method also permits a consideration of the problem as to whether, for example, three groups of 4 closely associated larvae are more or less aggregated than one looser group of 12 larvae.

A selected dandelion or cabbage leaf was supported by a wire attached along the mid-rib on the upper surface and kept fresh by setting up in a tube of water. The outline of the leaf together with the main veins was traced and the leaf was then mounted horizontally in a stand. The leaf was temporarily inverted and larvae were released evenly over the underside. The larvae were restricted by means of a hot wire to the leaf or, in the event of falling off, to the area immediately below it. Just above the leaf a sheet of plate glass was set up and at thirty-minute intervals the middle points of the individual larvae were plotted on the glass using a simple eyepiece system to obviate parallax. These plots were then permanently recorded on tracing paper and the relationship with the leaf could be easily reproduced by placing the tracing over the previously traced outline using marked reference points. Each experiment was run for five hours.

Simultaneous cultures of solitary and crowded larvae were set up and the above experiment was repeated at intervals throughout the period of their development. Larvae representative of the average stage of development were selected, care being taken to avoid the quiescent periods when the larvae were moulting. This latter factor influenced the times at which the experiment was carried out. Forty-five larvae approximately 2·7 mm. long were released at a density of one per 215 square mm., but as the larvae grew the density was decreased. Observations showed that, although the larvae were subsequently

considered in terms of area, equivalent conditions for aggregation could not be simply provided by a proportionate increase in the leaf area due to a pronounced " edge effect ". Larvae tended to aggregate in the edge region round the leaf and the number then depended on the circumference. Thus, as the larvae increase in length, to maintain the same density the leaf edge would have to be increased in proportion to larval length, while the area of the leaf would increase as the square of the larval length. It is not possible to fulfil these experimental requirements with normal undamaged leaves. The edge effect seemed to be of greater importance in the final aggregation and in the experiments the relation between the larval length and the leaf circumference was kept approximately to the same order, but when considering different stages of development the complexity of the situation must be kept in mind.

Observations on *P. gamma* larvae showed that an orbit of influence could be assumed for each larva, being approximately equal to a circle of diameter of one and a half times the resting length of the larvae. Disturbed larvae would remain attached to the substrate by their anal prolegs and would describe an arc with the free head end of the body from directly in front to immediately behind its own tail. Any other larva lying within this orbit would thus come into contact with the active disturbed larva. To include both active and resting larvae a working orbit as given by a circle of diameter of one and a quarter times the mean length of the larvae was applied to all larvae.

For each experiment larvae of approximately the same size were selected and their mean length was determined. Circles of appropriate diameter were then described about position points on the ensuing records. Where the circles did not overlap, the larvae were assumed to be having no effect on each other at the time of the observation. Where the circles did overlap, however, the larvae could have influenced each other and the greater the degree of overlap the greater the degree of effective aggregation. The level of aggregation of all the larvae could thus be considered in terms of the total area covered by the circles. When there were no larvae in contact with their neighbours, and accordingly no overlap of the circles, the total area would be maximal, but with closer grouping there would be overlap of the circles and the total area covered would be reduced. The amount of the reduction as caused by the aggregation when compared with the maximum possible area was expressed as a percentage and was used as an index of the level of aggregation. The results are given in Table II.

In Table II, *Series 1*, it can be seen that within one hour the crowded culture three days old had settled down to a level of aggregation that persisted throughout the experiment. The seven-days-old solitary culture, however, were much more variable ; neither did they settle down so well within the six-hour period of the experiment nor attain the level of aggregation shown by the crowded culture. A similar trend was shown when this experiment was repeated when the solitary culture was nine days old. It was observed that the solitary larvae were much more easily disturbed by other larvae and were more irritable in their reaction than were the crowded cultures. These experiments show, by the difference between the levels of aggregation of the solitary and the crowded cultures, that after three days the latter had been conditioned to some extent in this respect.

In *Series* 2 the larvae were released at a higher rate in terms of their size per unit area than in *Series* 1, so that the two are not comparable. However,

TABLE II.—*Degree of aggregation with time of dispersed larvae of* P. gamma (*for details see text*).

Series 1 : *Dandelion.*

Age in days.	Previous condition of culture.	Number of larvae.	Time (hours) ½	1	2	3	4	5	6
3	Crowded	45	0·5	2·8	2·3	3·1	2·8	2·7	.
7	Solitary	45	0·7	1·1	.	1·2	1·8	0	2·1
9	,,	20	1·3	0	1·2	1·4	1·4	.	2·4

Series 2 : *Purple Sprouting Broccoli.*

Age in days.	Previous condition of culture.	Number of larvae.	Time (hours). ½	1	2	3	4	5
14	Crowd	45	9·3	10·1	11·8	7·8	11·5	11·9
13	Solitary	45	9·2	7·7	4·3	7·1	8·0	7·8
20	Crowd	40	.	.	.	27·0	28·5	25·7
21	Solitary	35	.	.	.	22·4	12·3	10·4
33*	Crowd	20	15·7	20·5	18·8	12·9	.	.
35*	Solitary	20	17·3	12·3	13·8	9·5	.	.

* It was not possible to carry the experiment on beyond 3 hours due to the extensive amount of leaf eaten during this period.

it can still be seen that the solitary cultures were not only more variable but did not aggregate to the same degree as did the crowded ones. It also appears that in the crowded cultures the tendency to aggregate increases during the earlier part of the larval life but falls off in the later stages. The increase might well be due to the conditioning effect of continued association, this being finally supplanted by a tendency of the individuals to separate, as has been frequently observed in larval aggregations in the field.

Comparative studies on the behaviour of solitary and crowded cultures in their later stages of development had earlier shown that the latter spent more time feeding than their solitary counterparts (Long, 1953). Measurement of the leaf areas eaten by the end of experiments with 33 and 35-day-old larvae confirmed this observation, the crowded culture eating 49·8 sq. cm. in three hours whilst the solitaries ate 29·5 sq. cm. A repetition of this experiment gave a similar result. The equivalent comparison in the early stages of development was not made, due to the fact that there was considerable variation in the depth to which the surface of the leaf was eaten through.

It was observed that, under the conditions of the experiments and under field conditions, certain facets of the aggregation behaviour were associated with feeding and appeared with the youngest larvae used. Larvae would not readily feed on either very old or young leaves but preferred those which were just fully formed. As a result the larvae tended to concentrate on the underside of the leaves in this more restricted zone of the food plant. As well as being negatively geotropic the larvae were also positively phototropic. This would cause a larva released at random on the underside of a horizontal leaf to walk towards the edge of the leaf where the light intensity was greater. Once having reached the edge the larvae would either crawl along the edge or along the leaf surface

close to it, generally remaining on the underside. Unlike *P. brassicae* larvae, which could be guided by the silk web, the direction of the movement in *P. gamma* appeared to depend on chance, though the region in which they wandered tended to be restricted by their phototropism. The subsequent meeting and aggregation of larvae also depended on chance, but the opportunities for this happening were greatly increased by the accumulation of larvae in certain edge areas of the leaf. From time to time individual larvae may stop wandering and settle down to feed, but more frequently two larvae would meet and stop. Secured by their hind pair of prolegs these larvae would react to each other's presence by sharp movements of the head and body, sometimes accompanied by regurgitation. After this the larvae generally began to feed either at individual sites close together or at a common feeding hole. Larvae thus settled were then joined by other larvae in a similar manner and so an aggregation would build up. Naturally settled larvae were disturbed on occasions by other larvae sufficiently to cause them to leave their feeding site and begin to wander, but such larvae frequently met another adjacent larva and soon settled again. This factor helped to counteract the tendency of larvae to eat their way away from each other, though this would be finally checked by the ensuing reduction of the leaf area. Under solitary conditions the wanderings of larvae were not stopped in this way, and accordingly feeding took place at greater intervals. The subsequent effect on the level of nutrition may partly account for the slower rate of development of solitary cultures. Solitary larvae were more irritable and when they met on being released together in the experiments they went through a more prolonged period of agitation than in the larvae from the crowded cultures. As a result of which they neither settled so quickly nor aggregated to the same degree.

Observations on an Aggregation of *P. gamma* in the Field.

An experiment was designed to test the behaviour of an aggregation of *P. gamma* larvae in the field. A continuous single row of agricultural mustard 18 in. high and 5 ft. long was isolated by a surrounding strip of bare soil 12 in. wide, which was in turn enclosed by a continuous 18-inch vertical strip of Windolite[2] 12 in. high, the remaining 6 in. being buried. The whole area was protected from birds and rain by a rectangular frame 4 ft. high roofed with Windolite and covered with half-inch mesh wire netting. The row was marked off with small pegs into ten 6-inch divisions.

Larvae were reared in several crowded cultures and when they were half grown, and at a stage where they were unable to climb a vertical barrier of Windolite, 500 were selected from the cultures and released on the central plant. At intervals observations were then made in which the larvae on the plants above each of the 6-inch divisions were counted and the nature of the dispersion was estimated.

From the point of release the larvae were forced to move outwards by the availability of space on the leaves of the plants and in this experiment the majority of the larvae moved to one side of the centre into division 6. This movement is illustrated in fig. 3, where it can be seen that by 24 hours after

[2] A proprietary brand of thin wire mesh coated with a continuous film of transparent plastic.

the release the larvae were still aggregated about the centre of the row. In divisions 5 and 6 groups of 1 to 9 larvae could be found on individual leaves. After two days the aggregation still persisted in the original form, but with the destruction of the leaves on the central plants the numbers of larvae on the individual leaves in divisions 5 and 6 had fallen to a maximum of 6 but up to 3

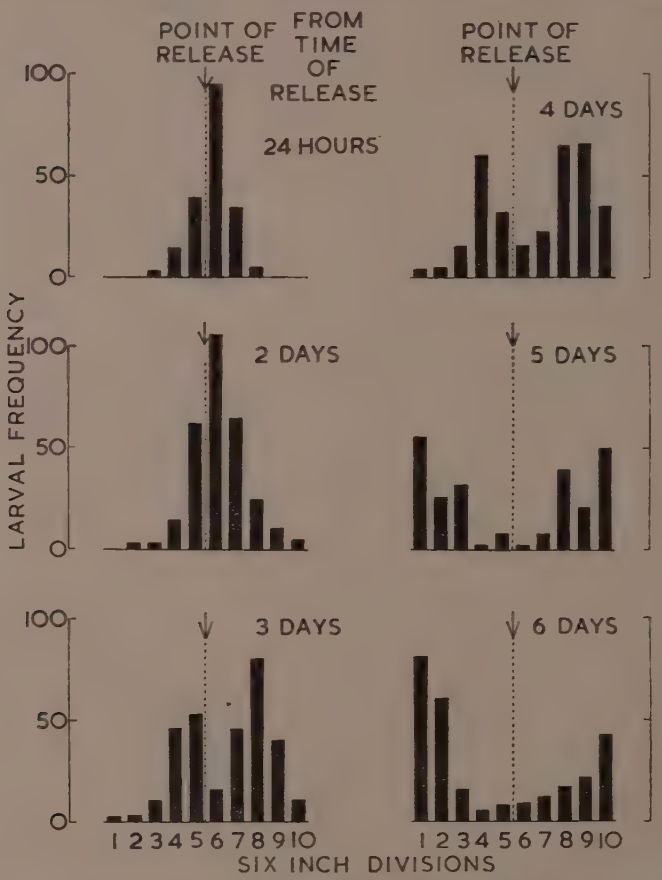

FIG. 3.—Movements of an aggregation of *P. gamma* larvae along a single row of agricultural mustard.

larvae per leaf could be found in divisions 4 and 7. After three days divisions 5 and 6 were nearly completely defoliated and the original aggregation was being split by an outward movement from this region of defoliation with the maximum numbers of larvae per leaf being found in divisions 4 and 8. With the majority of the larvae in divisions 6–10 this end of the row was defoliated first and larvae began to move back towards the other end, and this became clearly apparent by seven days.

After seven days 274 larvae were recovered and released on a fresh row of mustard. Twenty-four hours later larvae had already spread along the entire length of the row, though an aggregation covered divisions 4–7. By 48 hours, however, this aggregation had largely dispersed and the population, though reduced by pupation, had spread fairly uniformly over the entire row.

These observations support the findings of the laboratory experiments in that larvae in the middle stage of development will both form and remain in loose aggregations, but towards the end of their development they will actively separate.

The Effect of the Number of Larvae in Association.

In previous experiments, where the effect of the population density on the larval duration was being studied, it was noted that the relationship between the numbers of individuals involved and the degree of effect was unlikely to be a linear one. Accordingly, an experiment was set up in which the numbers of larvae reared in different cages followed the geometric series of 1, 3, 9, 27 and 81. A number of replicates were set up for each component of the series and the densities in each cage were maintained throughout the period of larval development by a reduction in the number of the respective replicates. The mean group colour as previously described (Long, 1953) and the mean larval duration were then determined and the results are shown in fig. 4.

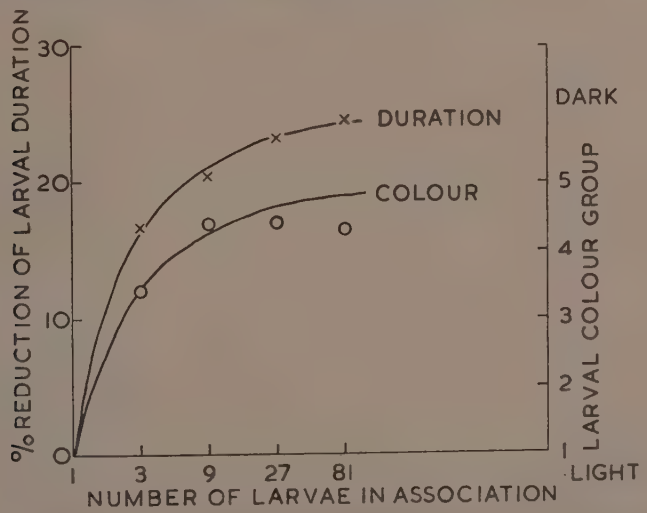

Fig. 4.—Effect of the number of larvae in association on larval duration and colour.

It can readily be seen that, under the conditions of the experiment, the relationship between the number of larvae and the duration was not a simple arithmetic one, nor was it linear for the geometric series used. This also appears to be true for the effect on the larval colour. Most of the effect in both cases occurred with the lower numbers of larvae. Thus 50 per cent. of the total effects produced with 81 larvae were obtained with two larvae.

A similar experiment was carried out to investigate the effect of the lower numbers of larvae using the arithmetic series of 1–6 larvae per cage. The results are shown in fig. 5, where again it can be seen that most of the effects were obtained with the lower numbers. On this occasion, however, effects

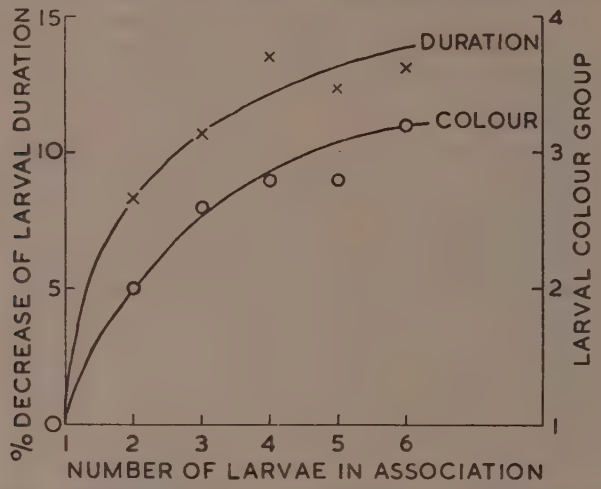

Fig. 5.—Effect of small numbers of larvae in association on larval duration and colour.

equivalent to the 50 per cent. values of the previous experiment were obtained with groups of 3–4 larvae and not with 2 as before. During the experiment the temperatures had been lower and the mean solitary larval duration was 54·4 days against the former 48·3 days. From this it appears that, within the range of temperature between these two experiments, raising the temperature increased these effects of the larval number.

Discussion.

In the aggregation of the two species of larvae studied differences in the mechanism and the pattern of behaviour in the aggregation are evident. *P. brassicae*, with its closely packed aggregations, depends on its characteristic habit of spinning a silk mesh on to the leaf surface to assist both the formation and maintenance of the aggregation. *P. gamma*, on the other hand, has no such guiding mechanism; the formation of the aggregation depends more on chance meetings and the individuals remain in a much looser association with their neighbours. In both cases, however, the ultimate factor of prime importance was that of larval contact. In *P. brassicae*, although wandering larvae were brought together by following the silk trails, their actual wandering was stopped by coming into contact with other larvae. In this case the sense of contact involved a thigmotactic response which led the larvae to settle in the closest contact with their neighbours and so form a tightly packed aggregation. This same sense of contact also played a vital part in the subsequent mass movements. Wandering larvae of *P. gamma* were also stopped by contact and

thus an aggregation would build up, but in the absence of a thigmotaxis a close aggregation was not formed. However, this response to contact was partly responsible for the maintenance of the aggregation. Larvae which tended to wander again, and so disperse the aggregation, were in a more densely populated region and accordingly the chances of further contact and their resettling was increased.

Larval contact was also responsible for enforcing a certain degree of activity in both species. As the larva with the shortest resting period would initiate the mass feeding movements of *P. brassicae*, the other larvae were induced to accept this faster rhythm and so the pace of activity was set at a higher level than would occur in solitary cultures. In *P. gamma*, too, a higher level of activity was set up. The larvae were motionless for two-thirds of the time, so that larvae in this state were most likely to be disturbed by other active larvae resulting in further movement or feeding. With associations of twelve larvae the total period of activity has been shown experimentally to be four times that of solitary larvae (Long, 1953). When, in this earlier experiment, the larvae which were normally associated were separated into solitary conditions it was observed that the level of activity did not drop, but the time spent feeding fell to nearly half its former level. It has been suggested (p. 430) that the aggregations were influenced by a change in larval behaviour as the result of continued association. The fact that in the earlier experiments the higher level of activity of associated larvae did not fall in isolation showed that larval contact had induced an intrinsic change in the behaviour of the individual in adopting this higher level of activity, and the tendency to aggregate at the same time may have been increased. The subsequent fall in the time spent in feeding would appear to follow from the lack of further larval contact in arresting free movement and inducing feeding.

The reduction of the larval duration and the darkening of the larvae of *P. gamma* were also shown to be associated with a sense of larval contact. Whether these effects are produced independently of the other factors discussed has yet to be determined. The question now arises as to whether larval contact or proximity can be recognised as such or whether it merely represents a mechanical disturbance resulting in increased activity. In locusts mechanically enforced activity certainly affects the colour (Husain and Mather, 1936), and in the caterpillars of *Exaereta ulmi* Schiff. mechanical stimuli were also claimed to be responsible for colour changes (Sharov, 1953*a*), though unfortunately the latter experiments were not supported by controls. With *P. gamma* the experiments described show that an association of two or three larvae can produce marked colour effects, whereas single larvae bred with large numbers of two other species namely, *P. brassicae* and *Diataraxia oleracea*, on a common food-plant failed to produce a significant effect. It is most unlikely that the single larvae were not subjected to repeated mechanical disturbances by the other larvae, so that it would seem that this factor in itself may not be sufficient. In the latter experiments or in those of Sharov it is not known whether there was a higher level of activity than would be found in their solitary counterparts, though this was most probable. The effect, therefore, need not depend on the mechanical stimuli itself, or the attendant increase in activity, but on some other factor directly or indirectly associated with larval contact, such as the feeding habit. Apart from crowding, other factors are known to affect the larval darkening,

such as absence of light (Faure, 1943, 1943*a*), different foods (Doull, 1953 ; Long, 1953) and, in locusts, carbon dioxide (Husain and Mathur, 1936*a*), but it has not been shown that any of these are necessarily responsible for the effects obtained in an association, only that the factors themselves are capable of producing a common end effect. It seems, therefore, that whilst there is evidence to indicate the potential effect of purely mechanical stimuli, the possibility has not been ruled out experimentally of there being some facet in the nature of the disturbance which would permit some form of larval recognition.

It is well known that large aggregations of *P. brassicae* may persist for some time in the field and experiment has shown that aggregations of *P. gamma* can also be maintained in the field. Although there are reports of mass outbreaks of *P. gamma* in the field (as in the Ormerod Reports for 1879, 1892 and 1897), such large numbers seldom occur in this country. It is, therefore, most significant that association of so few larvae as two or three can have such far reaching effect. These observations in the laboratory are in accordance with the field observations of Sharov (1953), where the colour form of *Exaereta ulmi* produced experimentally by larval association was found in the field on branches where there were two or three larvae. This renders of some consequence even slight tendencies to aggregate, as only a few larvae need be involved. The full implications of sub-social aggregation in the field have yet to be revealed, as studies have been chiefly carried out in the two insect orders of Orthoptera and Lepidoptera. From work not yet published the author has observed that effects may be produced in *Stollia fabricii* Kirk (Hemiptera), whilst Utida (1954) has shown that similar extensive effects may be obtained in the Cow-pea Weevil, *Callosobruchus quadrimaculatus* (Coleoptera). It seems probable, therefore, that the whole subject of aggregation may prove to be of general biological importance. These studies on Lepidoptera have shown the intricacies that occur in just two species, but they also indicate, through the small numbers of individuals that need be involved, the enormous potentiality of this factor in nature.

Summary.

Studies have been made on aspects of behaviour involved in the formation and maintenance of the tightly-packed aggregations of *P. brassicae* larvae and the looser associations of *P. gamma* larvae. The process was assisted in the former species by the guiding effect of the silken trails spun by the larvae, whereas contact between the larvae of *P. gamma*, whilst depending on chance, was assisted by the restrictive effect of their preference in the age of the food leaves and by a strong phototropism which would lead larvae towards the same region of a leaf.

In both species a sense of larval contact was shown to be of prime importance. *P. brassicae* larvae have a resting area away from the feeding site and this factor was responsible for the setting up of a mass feeding rhythm by keeping the larvae together in their movements to and from the feeding site. In *P. gamma* larval contact has been shown not only to be responsible for aggregation but also for the production of darker larvae and the reduction of the larval duration, which were amongst the effects of larval association previously reported (Long, 1953). The larvae were shown to be very sensitive to contact in

producing these effects, as the greater part of them could be produced by associations of two or three larvae.

The mass feeding rhythms of *P. brassicae* and the extra stimulation caused by repeated larval contact in *P. gamma* induced a higher level of activity which could persist under solitary conditions.

The ability of *P. brassicae* and *P. gamma* to aggregate at different stages throughout the larval period was studied; the tendency of larvae to separate in the later stages and some of the factors involved were shown. These results were supported by observations in the field.

ACKNOWLEDGEMENTS.

I wish to express my gratitude to Miss E. J. Nash for her unfailing assistance in the exacting routine task of breeding large numbers of caterpillars under experimental conditions. I also wish to thank Dr. C. B. Williams for his encouraging interest and for reading this manuscript.

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